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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS AND MATERIALS USING SIGNALING PROBES

(57) Abstract: The present invention relates to methods of isolating cells or generating cell lines using signaling probes that produce a signal upon hybridization to a target sequence. Other methods that utilize the signaling probe include methods of quantifying the level of RNA expression, methods for identifying genetic recombinational events in living cells and methods of generating a transgenic animal using the isolated cells. The invention also provides protease probes. Signaling probes and protease probes that form stem-loop structures, three-arm junction structures, and dumbbell structures are provided.

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US05/05080

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
  2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
  3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1,25,26,33 and 51-75
- Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US05/05080

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
IPC: C12Q 1/68( 2007.01);C07H 21/02( 2007.01),21/04( 2007.01) C12N 1/12( 2007.01),5/00( 2007.01)		
USPC: 435/6,252.1,325;536/23.1,23.4		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/6, 252.1, 325; 536/23.1, 23.4		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Medline		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PGPB, USPT, USOC, EPAB, JPAB, DWPI		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,692,965 B1 (SHEKDAR et al.) 17 February 2004 (17.02.2004), abstract, columns 1-18, Examples 1-3, claims 1-33.	1, 25, 26, 33, 51-75
X	US 6,485,901 B1 (GILDEA et al.) 26 November 2002 (26.11.2002), Figures 1A-8, columns 1-23, Examples 1-21.	1, 25, 26, 33, 51-75
X	US 5,866,336 (NAZARENKO et al.) 02 February 1999 (02.02.1999), Figures 1A-26, columns 1-44.	1, 25, 26, 33, 51-75
P	US 6,743,581 B1 (VO-DINH) 01 June 2004 (01.06.2004), Figures 1A-8, columns 1-35, Examples 1-9.	1, 25, 26, 33, 51-75
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
<b>* Special categories of cited documents:</b>		
"A" document defining the general state of the art which is not considered to be of particular relevance	"I" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	
"E" earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	
"P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 26 October 2006 (26.10.2006)		Date of mailing of the international search report 18 DEC 2006
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201		Authorized officer Amber D. Steele Telephone No. 571-272-1660

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US05/05080

## BOX III. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

Group I, claim(s) 1, 25-26, 33, 51-75, drawn to a method of isolating cells that potentially express RNA.

Group II, claim(s) 2-4, 25-33, 35-37, and 51-75, drawn to a method of isolating cells expressing RNA.

Group III, claim(s) 5, 12-15, 17, 25-26, 33, 35-37, 51-75, drawn to a method of isolating cells expressing RNA with two signaling probes.

Group IV, claim(s) 6-10, 12-15, 17, 25-33, 35-37, and 51-75, drawn to a method of isolating cells expressing two or more RNAs with two signaling probes.

Group V, claim(s) 11-15, 17, 25-33, 35-37, and 51-75, drawn to a method of isolating cells comprising DNA.

Group VI, claim(s) 16, 25-26, 33, 37, and 51-75, drawn to a method of isolating cells with RNA under the control of a conditional promoter.

Group VII, claim(s) 18-23, 25-33, 35-37, and 51-75, drawn to a method of isolating a plurality of cells.

Group VIII, claim(s) 24-33, 35-37, and 51-75, drawn to a method of isolating two or more RNA expression libraries.

Group IX, claim(s) 34, 37, and 51-75, drawn to a method of isolating cells with reduced protein expression.

Group X, claim(s) 38 and 51-75, drawn to a method of identifying a compound that activates a conditional promoter.

Group XI, claim(s) 39 and 51-75, drawn to a method of obtaining an RNA.

Group XII, claim(s) 40 and 51-75, drawn to a method of quantifying expression of RNA.

Group XIII, claim(s) 41-42 and 51-75, drawn to method of identifying a compound.

Group XIV, claim(s) 43 and 51-75, drawn to a method of identifying an RNA that modulates RNA expression.

Group XV, claim(s) 44-45 and 51-75, drawn to a method of identifying a genetic recombinatorial event.

Group XVI, claim(s) 46-49, drawn to a cell.

Group XVII, claim(s) 50, drawn to a method of making a transgenic or chimeric animal.

Group XVIII, claim(s) 76-98, drawn to a probe.

Group XIX, claim(s) 99-102, drawn to a DNA construct, vector, and a cell.

Group XX, claim(s) 103-114, drawn to a library of mammalian cell lines.

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Group XXI, claim(s) 115, drawn to a method of identifying a compound that enhances detection of targets.

Group XXII, claim(s) 116, drawn to a method of identifying a compound that mediates or improves introduction of signaling probes.

The inventions listed as Groups I-XX do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The common technical feature is a cell that can be isolated via a signaling probe. A cell that can be isolated via a signaling probe is known in the art.

Specifically, Shekdar et al. (U.S. Patent 6,692,965 B1 issued February 17, 2004) teach transfecting cell lines with DNA constructs, cells that have a first and/or second molecular beacon (e.g. signaling probes) that fluoresce upon hybridization to RNA, isolation of cells, generating cell lines, and generating transgenic mice (please refer to columns 1-18 and Examples 1-3). Therefore, a cell that can be isolated via a signaling probe is known in the art.